Novel nitrated derivatives of 5,8-diazabenzo[c]phenanthrene and 9,14-diazadibenz[a,e]acephenanthrylene: new classes of potent mutagenic compounds

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We report the synthesis of 4-nitro-5,8-diazabenzo[c]phenanthrene (4-NDBP) and 11-nitro-9,14-diazadibenz[a,e]acephenanthrylene (11-NDDA) and the remarkable mutagenic activity of the latter. These two compounds and their non-nitrated parents, 5,8-diazabenzo[c]phenanthrene (DBP) and 9,14-diazadibenz[a,e]acephenanthrylene (DDBP), were screened in Ames plate incorporation assays against Escherichia coli WP2uvrA and Salmonella typhimurium TA98 both in the presence and absence of S9 liver fraction from Aroclor 1254-induced rats. None of the four compounds were cytotoxic up to the limits of their solubility and none showed mutagenic activity in E.coli WP2uvrA, which suggested that any such activity they may have had was not mediated via a base substitution mechanism. DBP and DDA also displayed a lack of activity in TA98 up to their precipitating doses (560 and 33.5 µg/plate, respectively). The two nitrated compounds, however, were genotoxic. 4-NDBP was active at a dose of 500 ng/plate, in the absence of S9, producing 80.0 ± 28.0 prototrophic organisms (equivalent to 44 revertants/nmol) and at 0.5 ng/plate, in the presence of S9, giving 147 ± 6.6 revertants (equivalent to 81 000/nmol) and allowed the description of this tetracycle as a potent mutagen. Much more striking was the activity of 11-NDDA: in the absence of S9 a dose of 8.0 ng produced 2000 revertants/nmol and, remarkably, in the presence of S9 80 pg produced the equivalent of 643 000 revertants/nmol. This makes the hexacyclic 11-NDDA the most potent mutagen to date, in the Ames procedures described here.

Introduction

The connection between exposure to environmental carcinogens and site-specific cancers has been recognized since observations that nasal cancers in workers preparing snuff were caused by powdered tobacco (Hill, 1761) and that scrotal cancers in child chimney sweeps were related to their exposure to soot (Pott, 1775). Since that time much work has been done to identify the many hundreds of components of soot (Kaden \textit{et al.}, 1979), coal tars (\textit{Wise \textit{et al.}, 1988a}) and Standard Reference Material (\textit{Wise \textit{et al.}, 1988b}). Diesel particulate has received much attention in this respect (\textit{Lunde and Bjorseth, 1977; IARC, 1983; Nardini and Clonfero, 1992; Vaca \textit{et al.}, 1992; Stocker \textit{et al.}, 1996}). However, contrary to the general assumption that these contaminants are carcinogenic due to the presence of DNA adducts, the major source of benzo[a]pyrene, pyrene and fluoranthene in diesel exhaust originates from the fuel which survives combustion (\textit{Collier \textit{et al.}, 1995}; \textit{Tancell \textit{et al.}, 1995a,b, 1996}).

The recognition that the diaza derivatives of carcinogenic aromatic hydrocarbons are often themselves mutagenic and carcinogenic (\textit{Pai and Ranadive}, 1965; \textit{Buu-Hoi \textit{et al.}, 1967, 1968}) led to our own work in this area (\textit{Bloomfield \textit{et al.}, 1986}; \textit{Upton, 1986}; \textit{Tucker \textit{et al.}, 1993a,b; Upton \textit{et al.}, 1998}).

Many of the anthropogenic sources of these polycyclic and polyzapoly cyclic aromatic carcinogens contain nitrated derivatives which, although present in much lower concentrations, are much more potent and direct-acting mutagens, appearing as a class to be the most potent mutagens in Ames reversion assays (\textit{Mermelstein \textit{et al.}, 1981; Tokiwa and Onishi, 1986}). They have been reported in urban air particulates (\textit{IARC, 1983; Nardini and Clonfero, 1992}), diesel exhaust particulates (\textit{Nakagawa \textit{et al.}, 1983; Crebelli \textit{et al.}, 1991; Sera \textit{et al.}, 1994; Enya \textit{et al.}, 1997}), indoor air pollutants from cooking, kerosene heaters and cigarette smoke (\textit{Tokiwa and Onishi, 1986}), soil from wood, coal and brown coal burners (\textit{van Houdt \textit{et al.}, 1986}), grilled foods (\textit{Kinouchi \textit{et al.}, 1986}) and combusted aircraft fuel (\textit{McCartney \textit{et al.}, 1986}). They have also been reported as being clastogenic in chromosome aberration assays (\textit{Whittington \textit{et al.}, 1997}) and to cause DNA strand breakage (\textit{Mitchelmore \textit{et al.}, 1997}).

These nitro derivatives are considered to arise from reactions of the parent carboxyles and heterocycles with nitrogen oxides, peroxyacetyl nitrate, ozone and free radicals generated in such mixtures (\textit{Pitts \textit{et al.}, 1978, 1985; Zielinska \textit{et al.}, 1987}). Several authors have demonstrated the facile nitration of such molecules at ambient (\textit{Holloway and Ball, 1993}) and sub-ambient temperatures (\textit{Zielinska \textit{et al.}, 1986}).

The rationale for our own work lies in the isosteric relationships between mutagenic benzo[c]phenanthrene (1) and its diaza analogue 5,8-diazabenzo[c]phenanthrene (2) and dibenz[a,e]acephenanthrylene (4) and its analogue 9,14-diazadibenz[a,e]acephenanthrylene (5, Figure 1) (IUPAC, 1979). A problem encountered while searching the literature for these compounds concerns the varying nomenclature used for each class of compounds. The 5,8-diazabenzo[c]phenanthrenes (2) have been described, correctly, as quino[3,4-3’,4’]quinolines and benzo[a][3,6]phenanthrolines (Partridge and Vipond, 1962; Parfitt, 1966). In the hexacyclic compounds, carbazole dibenz[a,e]acephenanthrylene (4) has been named, incorrectly, as dibenzo[b,e]fluoranthene (\textit{Kaden \textit{et al.}, 1979}; \textit{Grimmer \textit{et al.}, 1983}; \textit{Wise \textit{et al.}, 1988a,b}) and its 9,14-diaza analogue (5) is named benzo[a]inden[1,2,3-de][3,6]phenanthrolines (Partridge and Vipond, 1962) and dibenz[e,f]inden[1,2,3-ij][2,7]napthyridine (\textit{Tucker \textit{et al.}, 1993b}). Benzo[c]phenanthrene (1) has been reported as a component of pyrolyses (\textit{Badger \textit{et al.}, 1962}), coal tar (\textit{Ferrand \textit{et al.}, 1964}), hydrocarbon oils (\textit{Dietz \textit{et al.}, 1956}), cigarette smoke (\textit{Orris \textit{et al.}, 1958}) and Standard Reference Material (\textit{Wise \textit{et al.}, 1988b}) and in emissions from foundries (\textit{Scheinberg, 1981}) and furnaces (\textit{Buck, 1983; Ramdhal and Moeller, 1983}). Its ubiquitous occurrence ensures it is found widely in the food chain (Mix

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Fig. 1. Structures of the benzo[c]phenanthrenes 1–3 and the dibenz[a,e]acephenanthrylenes 4–6.

et al., 1980; Vassilaros et al., 1981; Vaessen et al., 1984). It has been reported as a carcinogen and mutagen (Flurry, 1964). Dibenz[a,e]acephenanthrylene (4) has been reported far less frequently, occurring in coal tar extracts (Wise et al., 1988a), Standard Reference Material (Wise et al., 1988b) and in aquifers (Villaneuva et al., 1991). Its mutagenicity has been demonstrated (Kaden et al., 1979) and its fluorescence spectrum (Colmsjo and Wise, 1986), chromatographic detection (Sander et al., 1994) and synthesis have also been reported (Cho and Zhou, 1996). The diaza derivatives of the two molecules (1) and (4), 5,8-diazabenzo[c]phenanthrene (2) and 9,14-diazadibenzo[a,e]acephenanthrylene (5) are tumorigenic in whole mice (Partridge and Vipond, 1962).

In our own work with these compounds we noted that attempts to introduce functional groups into these heterocyclic nuclei, after their assembly, had not been described. Our need to have such functionalized nuclei is because these molecules are part of a targeting programme designed to enhance the specificity of the compounds by exploiting the ‘message-address’ concept (Portoghese, 1989; Portoghese et al., 1990) which we have successfully exploited with opioid ligands (Maguire et al., 1993). We have described nucleophilic substitution in these compounds (Upton, 1986; Upton et al., 1998) and their direct nitration is described for subsequent elaboration in our synthetic programme. In view of the reported genotoxic properties of this class of nitrated polyazapolycycles referred to earlier, we considered it judicious to evaluate these two compounds for such activity. It has been observed that these nitrated heterocycles are of particular interest as their genotoxic properties have not been extensively characterized (Holloway and Ball, 1993) and they have received little attention as pollutants due to a lack of authentic reference materials (Wise et al., 1988b). We regard these molecules as useful and authentic reference standards as well as the starting point for further elaboration.

Materials and methods

Chemistry

NMR spectra were recorded using a Jeol GX270MHz FT spectrometer (1H at 270 MHz and 13C at 67.8 MHz). IR spectra were recorded with a Perkin-Elmer 782 infrared spectrophotometer. Gas chromatograms were obtained from a Chrompack CP 9001 machine using a 10 m Chrompack CPSil5 column. The initial oven temperature of 150°C was increased at a rate of 15°C/min and then held at 260°C for 10 min using a flow rate of helium of 1 ml/min through the column and sample volumes of 1 µl of a 1 mg/ml solution (in HPLC grade CHCl3). Melting points are uncorrected. Thin layer chromatography was performed on silica gel 60 F254 plates from Merck (Poole, UK); plates were visualized by illumination at 254 nm. t-Histidine, boitin, methyl methanesulphonate, 2-aminofluorene, β-nicotinamide adenine dinucleotide phosphate and d-glucose-6-phosphate disodium salt were purchased from Aldrich-Sigma Chemical Co. (Poole, UK). 5,6-Diazabenzo[c]phenanthrene (2) and 9,14-diazadibenz[a,e]acephenanthrylene (5) were prepared as previously described (Upton, 1986). 4-Nitro-5,8-diazabenzo[c]phenanthrene (3) was prepared from (2) by nitration with potassium nitrate in sulphuric acid at 80°C for 3 h to give the required nitrophenanthrene (5) (0.116g, 19%) as pale yellow needles, m.p. 173–175°C (ethanol). 11-Nitro-9,14-diazadibenzo[a,e]acephenanthrylene (6) was prepared from (5) by nitration with nitric acid in sulphuric acid 70°C for 3.5 h to give the acephenanthrylene (6) (0.034g, 12%) as yellow needles, m.p 245–247°C (xylene). Both (3) and (6) were purified to chromatographic homogeneity and their physical data (NMR, MS, HRMS and/or elemental analysis) was consistent with the proposed structures. The chromatographic retention times on a CPSil5 column for these four compounds (2, 3, 5 and 6) were 2.6, 6.2, 4.1 and 7.75 min, respectively.

Mutagenicity assays

The test organisms were grown from refrigerated samples to a concentration of 1–2×10⁸ organisms/ml in Nutrient Broth (Difco, West Molesey, UK), harvested and suspended in phosphate buffer. Spot tests were performed (Bridges, 1972) as an initial, rapid screen for a large number of structurally related benzenanthrenes. Results were all negative and are not displayed in this paper. Standard plate incorporation assays with Salmonella typhimurium TA98 and tryptophan-dependent Escherichia coli were used (Green and Muriel, 1976; Maron and Ames, 1983). To 2 ml of top agar were added 100 µl of fresh overnight culture, 10 µl of test chemical in DMSO and 50 µl of top agar (as needed) and mixed gently before pouring onto minimal agar plates. Duplicate experiments were carried out for each compound, using three plates at each dose used. Negative controls containing bacteria and solvent (with and without S9) were prepared along with positive controls containing an appropriate diagnostic mutagen [methyl methanesulphonate (1 µl) for E.coli and 2-aminofluorene (15 µg) for TA98]. The plates were incubated at 37°C for 48 h and revertant, prototrophic organisms counted; the results are shown in Tables I and II. The S9 liver fraction was derived from Acrorol 1254-induced rats and acquired from Inveresk Research (Edinburgh, UK).

Results

Chemistry

4-Nitrobenzo[c]phenanthrene (3) was identified as the mononitrated heterocycle by mass spectrometry (m/z 275, M⁺ 100% abundance, with losses characteristic of a nitro-containing compound: [M-30]⁺ for N-O and [M-46]⁺ for -NO₂. A high resolution accurate mass was obtained from the electron impact spectrum). Elemental analysis and infrared characteristics were also consistent with this assignment. The position of the substituent was determined by proton spectroscopy. Gas chromatography confirmed the product to be a single entity for screening purposes. Similar arguments apply to assignment of the substitution position in the acephenanthrylene (6), confirmed as a mononitro compound by EIMS and accurate mass determinations. The simple proton spectrum of this unsubstituted parent heterocycle was also disrupted by a loss of symmetry with introduction of the nitro group. In order to interpret possible differences in the biological activity of these benzo[c]phenanthrenes in terms of physicochemical properties, we cited their theoretical log P values (Upton et al., 1998), obtained using Lab Beta 3 software produced by the Advanced Chemical Development (ACD) Co. These values have been queried and so they have been recalculated using different packages. The two packages used were those which were most highly rated in a recent survey of log P calculation methods (Mannhold and Dross, 1996). They recommended KOWWIN and ClogP and the results from those methods are recorded here. The compounds cited were 5,8-diazabenzo[c]phenanthrene and 9,14-diazadibenzo[a,e]acephenanthrylene [compounds (2) and (5) of this report] and 3,10-dichloro- and 2,3,10,11-tetra-
Table I. Mutagenicity results using 5,8-diazabenzo[c]phenanthrene (2), its 4-nitro derivative (3) and 9,14-diazadibenzo-\[a,e\]acephenanthrylene (5) and its 11-nitro derivative (6) against *S.*typhimurium TA98 in the plate incorporation assay

| Sample | In the presence of S9 | | In the absence of S9 | |
|--------|----------------------|------------------|-------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| 2      | Dose (µg)\(^{a}\)    | 560              | 56                | 0.56             | 560              | 56                | 0.56             | 560              | 56                | 0.56             |
|        | Revertant organisms\(^{b}\) | 48.3 (±11.6)  | 62.0 (±5.5)     | 57.0 (±4.6)     | 500              | 500               | 5.0              | 500              | 500               | 5.0              |
| 3      | Dose (ng)             | 500              | 500               | 50               | 500              | 500               | 5.0              | 500              | 500               | 5.0              |
|        | Revertant organisms   | 376.6 (±35.1)    | 201.7 (±22.5)    | 171.7 (±22.5)   | 137.0 (±14.9)    | 147.0 (±16.6)    | 425.3 (±63.3)    | 33.3 (±6.4)      | 29.0 (±2.6)      | 37.0 (±7.2)      |
| 5      | Dose (µg)             | 33.5             | 6.7               | 0.67             | 33.5             | 6.7               | 0.67             | 33.5             | 6.7               | 0.67             |
|        | Revertant organisms   | 47.0 (±4.6)      | 49.7 (±2.5)      | 49.3 (±9.6)     | 33.0 (±14.1)     | 34.3 (±6.0)       | 36.7 (±2.8)      | 47.0 (±4.6)      | 49.7 (±2.5)      | 49.3 (±9.6)      |
| 6      | Dose (ng)             | 800              | 800               | 8.0              | 8.0              | 8.0               | 8.0              | 8.0              | 8.0               | 8.0              |
|        | Revertant organisms   | 489.3 (±27.3)    | 183.3 (±9.5)     | 162.3 (±13.7)   | 137.7 (±7.5)     | 147.3 (±9.7)      | 275.3 (±65.8)    | 275.3 (±65.8)    | 17.0 (±3.6)       | 17.0 (±3.6)       |
| 2AF\(^{c}\) | Dose (µg)             | 15               | 15                | 23.6 (±2.1)     | 15               | 15                | 23.6 (±2.1)     | 15               | 15                | 23.6 (±2.1)     |
|        | Revertant organisms   | >1600            | >1600             | >1600            | >1600            | >1600             | >1600            | >1600            | >1600             | >1600            |
| DMSO alone\(^{d}\) | Dose (µl)             | 0                | 5                 | 20               | 0                | 5                 | 20               | 0                | 5                 | 20               |
|        | Revertant organisms   | 33.5 (±1.5)      | 40.5 (±1.5)      | 41.0 (±5.0)     | 40.0 (±2.0)      | 40.0 (±2.0)       | 41.0 (±5.0)      | 40.0 (±2.0)      | 40.0 (±2.0)       | 40.0 (±2.0)      |

\(^{a}\) The first dose for each compound represents the precipitating dose (as described by Gatehouse et al., 1994).

\(^{b}\) Result of triplicate determinations at each dose used; a uniform background bacterial lawn of growth was observed in all cases.

\(^{c}\) 2-Amino-thioarene (2AF) is used as a diagnostic, positive mutagen.

\(^{d}\) The solvent for these procedures, dimethylsulphoxide (DMSO), is shown to be without mutagenic properties.
Table II. Mutagenicity results using 5,8-diazabenzo[c]phenanthrene (2) and 9,14-diazadibenzo[a,e]acephenanthrylene (5) and their mononitro derivatives (3) and (6) against E.coli WP2uvrA in the plate incorporation assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>In the presence of S9</th>
<th>In the absence of S9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Dose (µg)a</td>
<td>560</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>7.33 (±2.5)</td>
</tr>
<tr>
<td>3</td>
<td>Dose (ng)</td>
<td>5000</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>4.0 (±2.6)</td>
</tr>
<tr>
<td>5</td>
<td>Dose (µg)</td>
<td>33.5</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>3.67 (±2.8)</td>
</tr>
<tr>
<td>6</td>
<td>Dose (ng)</td>
<td>800</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>2.67 (±0.6)</td>
</tr>
<tr>
<td>MMSb</td>
<td>Dose (µl)</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>132.3 (±23.1)</td>
</tr>
<tr>
<td>DMSOc</td>
<td>Dose (µl)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Revertant organisms</td>
<td>7.7 (±1.5)</td>
</tr>
</tbody>
</table>

As in Table I.
As in Table I.
MMS, methyl methanesulphonate, was used as a diagnostic, positive mutagen.
Negative control plate to determine spontaneous reversion rates.

methyl-5,8-diazabenzo[c]phenanthrene. The values obtained previously were 3.18, 5.13, 4.68 and 3.68, respectively. Corresponding values after recalculation (KOWWIN value first) are 3.47 and 3.50, 5.23 and 5.55, 4.11 and 4.23, 3.11 and 3.55, respectively. Thus the ACD method appears to give results comparable with these other packages.

Mutagenicity assays

The results of screening compounds (2), (3), (5) and (6) in plate incorporation assays are shown in Table I, using prescribed protocols for such preliminary screening (Gatehouse et al., 1994) with S.typhimurium TA98 as test organism. Mutagenicity of a sample was attributed if it was capable of producing at least double the number of spontaneous revertants seen, a definition previously accepted (Nardini and Clonfero, 1992). Table I records the results for the four compounds pertinent to this report. At all dose levels used, the two agents appeared to be non-toxic to TA98, as a uniform background bacterial lawn was seen in all plates tested. With both agents mutagenic activity is higher in the presence of the Aracor-induced S9 liver fraction. The bacteria were shown to retain their susceptibility to mutagenic agents by the inclusion of 2-aminofluorene, the diagnostic mutagen for TA98 in the presence of S9, when very large numbers of revertants were noted (>1600/plate). The solvent for the assays (DMSO) was shown to have no mutagenic activity itself.

The remarkable potency of these nitro compounds is evident when the number of revertants/nmol is calculated: at the lowest dose at which activity is seen, these figures are 81 000 and 643 000 for (3) and (6), respectively. This figure of 643 000 appears to be the highest one reported for mutagenicity in the Ames test. Prior to this report the most potent mutagenic mononitro compound was 3-nitrobenzanthrone [Enya et al., 1997, who recorded activity at 208 000 revertants/nmol and compared it with 1,8-dinitropyrene (257 000 revertants/nmol), the most active direct acting mutagen in TA98 in the literature]. When the four compounds (2), (3), (5) and (6) were screened in similar assays using tryptophan-dependent E.coli WP2uvrA as test organism (Table II), no mutagenicity was detected for any compound up to the precipitating dose.

Discussion

Chemistry

Electrophilic substitution in these 5,8-diazabenzene[c]phenanthrenes and 9,14-diazadibenzo[a,e]acephenanthrylenes has not been described previously and we required the functionalized nuclei for further elaboration: nitration was selected for the work here. Regarding the parent (2) as a quinoquinoline one would have predicted mononitrination in the carbocyclic ring at positions equivalent to 5 and 8 of quinoline [C-1 and C-4 in (2)]. The configuration of the benzophenanthrene (2) almost certainly precluded nitration at C-1, in the fjord region, on steric grounds and this was borne out in practice. Similarly, in the hexacyclic acephenanthrylene (5), predicted nitration in the least deactivated ring, that distal from the heterocyclic rings, also occurred.

Reaction conditions chosen for nitration of the benzophenanthrene (2) and dibenzacephenanthrylene (5) were those which produced minimal polynitrination. The presence of polynitrated products may have produced skewed results in the mutagenicity assays as several authors have reported that dinitro and polynitro derivatives are more mutagenic than their corresponding mononitrated counterparts: 3,6-dinitrocarbazole was much more potent than 3-nitrocarbazole (Holloway and Ball, 1993) and 1,6-dinitro and 1,8-dinitropyrenes are orders of magnitude more active than mononitropyrenes (Mermelstein et al., 1981; Pederson and Siak, 1981; Rosenkranz and Mermelstein, 1983).

Mutagenicity

Within nitrated aromatic polycycles generally there does not appear to be a consistent, predictive pattern concerning the effects of S9 on mutagenicity. Several authors have described higher mutagenic capability in such compounds in the absence of S9; e.g. 3-nitrofluorantheine (van Haeringen et al., 1993; Ball et al., 1995), 3,6-dinitrocarbazole (Holloway and Ball, 1993) and 2,7-dinitrofluorene (McCoy et al., 1981b; Tokiwa et al., 1981). Other authors have noted a reversal of this situation and seen enhanced mutagenicity in the presence of S9; e.g. 1-nitropyrene (Pitts et al., 1982), 5-nitroacenaphthene (Tokiwa et al., 1981; Rosenkranz et al., 1982), 2-nitrochrysene, 3-nitropyrene and 6-nitrochrysene (Nilsson et al., 1981) and 6-nitrobenzo[a]pyrene (Tokiwa et al., 1981). Yet other authors report results run solely in the absence of activating enzyme with no comparative data; e.g. 2-nitronaphthalenes (McCoy et al., 1981a), nitroazabenzo[a]pyrenes (Sera et al., 1994) and dinitropyrenes (Tokiwa et al., 1985).

The S9 liver fraction used in the Ames test has a complement of reducing and oxidizing enzymes mediating xenobiotic
metabolism. Thus, S9 may not only facilitate nitro reduction to amine and generation of the subsequent mutagenic cascade, but will also, simultaneously, be pursuing other metabolic pathways. These may give rise to oxidized products (C-epoxides, diols and phenols) whose presence may enhance the reactivity of labile hydroxylamines, N-acetylhydroxylamines and their ephemeral free radical derivatives. Alternatively, these oxidized nitrated polycycles may be mutagenic per se, as they are more reactive electrophiles and may exhibit higher mutagenic activity than the nitrated substrate itself. It has been demonstrated that some hydroxynitrocarbazoles are more active than their nitro counterparts (Holloway and Ball, 1993) and recently it has been shown that hydroxylated aromatic amines produce reactive free radicals at higher concentrations than are produced by the unsubstituted amines themselves (Brennan and Schiestl, 1997). Other authors have discussed these issues (El-Bayoumy and Hecht, 1982).

The variation in mutagenic capabilities within a regiosomeric series must reflect the ease of accessibility of the xenobiotic to the enzyme’s active site and this has been demonstrated with fluoranthenes, pyrenes and benzo[a]pyrenes (Rosenkranz and Mermelstein, 1983) and 7-benzanthrones (Enya et al., 1997). For this reason it would seem useful to include experiments run both in the absence and presence of S9. In the cases of 5,8-diazabenzo[c]phenanthrene (2), 9,14-diazadibenzo[a]acene (5) and their mononitro derivatives (3) and (6), respectively, these appear to be non-mutagenic in E. coli WP2uvrA (Table II), both in the presence and absence of S9. This is somewhat predictable behaviour, as E. coli is usually unaffected by frameshift-specific mutagens, the usual mechanism mediating the mutagenic activity of nitrated polycycles, responding, instead, by reversion to tryptophan independence via base substitution at A-T pairs. This supports the idea that mutagenic capability in this series is related to a direct-acting mechanism and restricted to the nitrated derivatives (3) and (6) themselves, as the unsubstituted parents (2) and (5) appear to be non-mutagenic in TA98 (Table I) and the introduction of the nitro group is the sole arbiter of mutagenicity in this series of compounds. Both nitro compounds are mutagenic in the absence of S9, reflecting direct activity in the cell, but of much lower potency than when tested in its presence. As referred to earlier, some authors screen compounds in the absence of S9 only: in this instance only the moderate activity of (6), at 2000 revertants/nmol, would have been discovered and its remarkable mutagenicity in the presence of metabolizing enzymes, which mimic the in vivo environment, would have remained unreported. This very high activity is noteworthy for another reason. In a review of mutagenicity in 60 nitro-containing aromatic polycycles, structure–activity relationships were described in which mutagenic activity in the series rose from very weak in bicycles to a maximum in tetracyclic compounds, with an abrupt decrease in activity in pentacyclic systems (Rosenkranz and Mermelstein, 1983). The 9,14-diazadibenzo[a]acene (6) is hexacyclic and supports our contention for the need for such potent compounds to be included in Standard Reference Material samples for environmental screening, as the vast majority of such standards are the tetracyclic fluoranthenes, acenaphthenylene, pyrenes and chrysenes.

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New classes of potent mutagenic compounds


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